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Identification and functional characterization of tumor-suppressive microRNAs with potential key roles in breast cancer progression and metastasis

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Downregulation of tumor-suppressive microRNAs in breast cancer (BC) triggers molecular disturbances underlying cancer initiation, disease progression and metastasis. However, key microRNAs players acting as vital breaks on BC aggressiveness, with crucial regulatory roles in cell migration, epithelial-mesenchymal transition (EMT), invasion, and metastasis, remain underinvestigated. Our goal was to uncover candidate tumor-suppressive microRNAs associated with metastatic potential of BC via an *in silico* approach, which combined data from microRNA profiling studies on cell lines relevant to BC invasiveness, as well as on primary vs. metastatic BC. A minimal set of candidate microRNAs was determined from the intersection of downregulated sets from multiple eligible studies, confirmed by qPCR, assessed for potential clinical relevance in BC and functionally characterized. Predicted and experimentally validated targets of these microRNAs were identified (MiRTargetLink) and subjected to gene ontology (GO), KEGG pathway, and cancer hallmark enrichment analysis. A microRNA-mRNA interaction network was constructed (MiRNet), and mRNA profiling data was integrated, in order to enable the identification of hub genes and upregulated microRNA targets mediating BC EMT, invasion, and metastasis. Three microRNAs emerged as plausible BC metastasis-related candidates: miR-141-3p, miR-200c-3p, and miR-205-5p. The downregulation of these microRNAs in cell lines with higher invasiveness was confirmed by qPCR, while an association with negative clinical features was also noted. Both members of miR-200 family demonstrated a correlation with the expression of key regulators of EMT in BC tissues. Hub genes from DE-microRNA-mRNA network showed enrichment in pathway and GO terms related to malignant phenotype, while “tissue invasion and metastasis” was one of the top enriched cancer hallmarks. These results support the hypothesized functional relevance of analyzed microRNAs in BC.

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microRNAs, invasion, EMT, metastasis, BRCA